mercaptoethanol (ME) and 10% human serum type AB. RPMI 1640 culture medium is known and used by those of skill in the art and can be obtained from a variety of vendors. Attachment A is a description of RPMI 1640 medium supplied by Invitrogen. A review of Attachment A will indicate that there are no ingredients in RPMI 1640 medium that are gel forming or that provide high viscosity to the medium. As disclosed at page 46, lines 1-3, in the peptide stimulated cell population, CD8+IFN-γ+ cells were enriched up to 40%.

The specification at page 47 under Example 2 describes a method for the enrichment of IFN- γ-secreting cells with the magnetic cell separation system, MACS, from peripheral blood mononuclear cells (PBMC) cultured in peptide MI 58-66 from Influenza virus matrix. As described at page 47, lines 1-7, the cells were cultured in media containing complete RPMI 1640 containing 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.3 mg/ml glutamine, 10 mM 2-ME and 10% human serum type AB. There is no high viscosity or gel forming component of the medium. As disclosed at page 48, lines 10-14, in the peptide stimulated cells, CD8+IFN-γ+ cells were enriched up to 41.6%.

Furthermore, all of Examples 4-8 describe methods of the invention performed in the absence of high viscosity or gel forming medium.

For Examples 4-8, see page 49, lines 1-14, which disclose that the culture medium for Examples 4-8 consists of RPMI 1640, 10% human AB serum, 1mM L-alanyl-glutamine, 100 U/ml penicillin/streptomycin, 0.05 mM 2-ME and 1 mM sodium-pyruvate. No high viscosity or gel forming components were present in the media used in Examples 4-8.

Applicants thank the Examiner for the courtesy of the telephone discussion on June 19, 2001. Pursuant to that phone call, Applicants invite the Examiner's attention to the reference Brosterhus, et al., 1999, *Eur. J. Immunol.* 29:4053-4059 attached as reference 4 on the Form PTO-1449 submitted along with the Supplemental Information Disclosure Statement both of which are submitted concurrently herewith.

Brosterhus et al. describe the enrichment and detection of antigen-specific CD4+ and CD8+ T-lymphocytes based on cytokine expression. In Brosterhus et al. antigen specific CD4+

and CD8+ T lymphocytes are subject to specific stimulation with peptides, proteins or complex antigen preparations to induce secretion of cytokines. After the stimulation period, an affinity matrix for IFN-γ was created on the cell surface using Ab-Ab conjugates directed against CD45 and IFN-γ (anti-IFN-γ-CD45) and the cells were allowed to secrete IFN-γ in culture for 45 minutes. Then, IFN-γ, relocated to the affinity matrix of the secreting cells, was stained with PE-conjugated IFN-γ-specific Ab and PE-labeled cells were enriched by MACS using anti-PE Ab microbeads. See Brosterhus et al. page 4053 under Section 2.1. As disclosed by Brosterhus et al., under the experimental conditions where the cells were stimulated with FLU 58-66, a significantly higher proportion of IFN-γ+ CD8+ cells were detected. See Brosterhaus et al. page 4053, right column. As disclosed by Brosterhus et al., under the experimental conditions where the cells were stimulated with influenza A virus preparation, a significantly higher proportion of IFN-γ+ CD4+ cells were detected. See Brosterhaus et al. page 4054, left column. In Brosterhaus et al. at page 4057, under Section 4.1, it is disclosed that the culture medium consisted of RPMI 1640, 10% human AB serum, 1 mM L-alanyl glutamine, 100 U/ml penicillin/streptomycin, 0.05 mM 2-ME and 1 mM sodium pyruvate. No high viscosity or gel forming component was present in the medium disclosed in Brosterhaus et al.

The Office Action alleges that Manz et at. establish the need for high viscosity media to practice the instant invention. This clearly has not been demonstrated and in fact, the specification and Brosterhaus et al. demonstrate that the presently claimed methods will work successfully in the absence of high viscosity or gel forming medium.

Therefore, Applicants submit that the presently claimed invention is enabled across its full scope. One of skill in the art following the disclosure of the application would be able to make and use the methods of the invention in the absence of high viscosity or gel forming medium and optionally, in the presence of high viscosity or gel forming medium, without any undue experimentation.

Therefore, Applicants respectfully request withdrawal of the Section 112, first paragraph rejection of claims.

Supplemental Information Disclosure Statement submitted February 16, 2001

Applicants respectfully request that the Examiner initial the form PTO-1449 submitted February 16, 2001.

CONCLUSION

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 212302000720. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: July 9, 2001

By: _____

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